REMARKS

Rejections Under 35 U.S.C. §102(e)

The Examiner rejected all of the pending claims as anticipated under 35 U.S.C. §102(e) by U.S. Patent No. 6,326,145 (the '145 patent"). Applicants respectfully traverse this rejection.

The Examiner, citing col. 12, lines 54-67 and col. 13, lines 1-52 of the '145 patent (Example 1), argues that the '145 patent discloses as method for achieving differential amplification of two different alleles in a mixture such that a first nucleic acid molecule having a first nucleotide present at a polymorphic site is amplified to a greater extent than a second nucleic acid molecule having a second, different nucleotide present at the polymorphic site. Citing other portions of the '145 patent the Examiner argued that the '145 patent describes a primer that forms a stem loop only when certain sequence are present in a target nucleic acid molecule that serves as template for extension of the primer. Applicants agree that the '145 patent describes a probe that can form a stem loop under certain conditions. However, the '145 patent does not describe a method for achieving differential amplification.

The '145 patent does not disclose differential amplification

The '145 patent does not disclose a method for achieving differential amplification of two different nucleic acid molecules. In particular, Applicants fail to understand how the Examiner has concluded that Example 1 (or any other portion) of the '145 patent discloses differential amplification. Example 1 discloses a PCR amplification in the presence of a probe ("Scorpion probe") that serves a means for detecting a nucleic acid molecule having a particular sequence, e.g., a particular allele. Fig. 13 of the '145 patent shows that the fluorescent signal produced by a Scorpion probe that matched the target allele increased as the PCR amplification reaction increased the amount of template DNA in the reaction. Applicants cannot see any suggestion that differential amplification occurred in the reaction described in Example 1. Indeed, Applicants cannot even see any suggestion that two different alleles were present in the

reaction described in Example 1. Applicants respectfully request that the Examiner explain, in detail, the basis for concluding that differential amplification takes place.

The stem-loop formed by the '145 probe is for detection, not differential amplification

The '145 patent describes a detection probe ("Scorpion probe") that is capable of forming a stem-loop when a selected sequence is present in a sample. The Scorpion probe includes a **signaling system** such a fluorophore and a quencher. The Scorpion probe also includes a **template binding region** that hybridizes to a template nucleic acid molecule. The Scorpion probe also has a **target binding region** that can participate in the formation of the stem of a stem-loop structure if the probe is extended to include a sequence complementary to the target binding region. Formation of the stem-loop prevents the flourophore form interacting with the quencher. Thus, formation of the stem-loop structure leads to fluorescence.

As the '145 patent explains, the Scorpion probe is a sensitive means for detecting a particular sequence. The Scorpion probe hybridizes to a sequence within a template nucleic acid molecule via the templates binding region. Extension of the Scorpion probe on the target will cause the incorporation into the probe of additional sequence. If extension of the probe creates a sequence that is complementary to the target binding region already present within the probe, then a stem-loop structure forms and a fluorescent signal is emitted. If extension of the probe creates a sequence that is NOT complementary to the target binding region already present within the probe, then the stem-loop structure does NOT form and NO fluorescent signal is emitted. Thus, it can be seen that Scorpion probe is a detection system, not a primer for differential amplification. In fact, the '145 patent explains (col. 5, lines 51-56) that the method described are detection methods that are used in conjunction with amplification methods (col. 5, lines 64-65).

These is nothing in the '145 patent to suggest that the Scorpion probe can cause differential amplification of two template nucleic acid molecules based on whether the target binding region of the probe matches a sequence within one or the other of the template nucleic acid molecules. Indeed, the '145 patent discloses just the opposite. Example 2 of the '145 patent

describes the use of two different Scorpion probes – one that matches the sequence present in the template nucleic acid molecule and one that does not match the sequence present in the template nucleic acid molecule. Each Scorpion probe was added to a PCR amplification reaction containing the template nucleic acid molecule. As shown in Fig. 14, the matched Scorpion probe (i.e., the one having a target binding region perfectly complementary to a sequence in the template nucleic acid molecule) generated a strong fluorescent signal while the mismatched Scorpion probe (i.e., the one having a target binding region that is NOT perfectly complementary to a sequence in the template nucleic acid molecule) did not generate a significant fluorescent signal. The '145 patent explains that "both amplifications were equally efficient" (col. 13, lines 58-60). This in not the result that would be observed if the Scorpion probe was causing differential amplification. If the Scorpion probes were causing differential amplification, the efficiency of the amplification reaction would depend on whether the matched or mismatched Scorpion probe was present in the amplification reaction.

The scorpion probes are not fully incorporated into the amplification product

As explained in the response filed February 23, 2005 at page 6 and as explicitly stated in the claims, one of the two primers used in the presently claimed methods includes "a 5' portion which, when incorporated into an amplification product, will upon further amplification yield products that form a stable stem-loop structure." The Scorpion probe of the '145 forms a stem loop, but is very different. As the '145 patent explains, the Scorpion probes include a blocking moiety that prevents the "tail region", which includes the 5' target binding region from being amplified (see col. 2, lines 54-67). Thus, the Scorpion probes do not contain a 5' region that is incorporated into the amplification product as required by the present claims.

It is clear that the '145 patent does not teach or suggest the presently claimed methods, all of which achieve differential (biased) amplification of nucleic acid molecules that differ in the nucleotide present at a polymorphic site. In view of this, Applicants respectfully request that the rejections under 35 U.S.C. §102(e) be withdrawn.

Enclosed is a Petition for Extension of Time with the appropriate fee. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: 16 NOV 2005

Anita L. Meiklejohn, Ph.D.
Reg. No. 35,283

Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

21207973.doc